



Advancements in Cancer Immunotherapy: A Comprehensive Review of Nanoparticle-Based Approaches

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ABSTRACT

Cancer immunotherapy has emerged as a promising approach to treating various types of cancer. Recent advancements in nanoparticle-based approaches have shown potential in enhancing the efficacy of immunotherapy. This review aims to provide a comprehensive overview of the current state of cancer immunotherapy, with a focus on nanoparticle-based approaches. We discuss the different types of cancer immunotherapy, including adoptive cell therapy, antibody-based targeted therapy, cancer vaccines, oncolytic viruses, and cytokine therapy. We also highlight the role of nanoparticles in improving the delivery and efficacy of these therapies. Additionally, here we explore the potential of nanoparticles in targeting antigen-presenting cells, delivering antibodies, and genes. Furthermore, we discuss the various types of nanoparticles, including PLGA, liposomes, dendrimers, virus-like particles, and hybrid particles, and their applications in cancer immunotherapy.

Keywords: Cancer immunotherapy, Nanoparticles, Targeted therapy, Vaccine delivery, Immunomodulation.

INTRODUCTION

Cancer is a serious public health issue worldwide, and the future burden of cancer is predicted to rise due to population growth and lifestyle changes. According to global cancer observatory statistics (GLOBOCAN), about 19.3 million new cancer cases were reported in 2020, with an estimated 10 million fatalities¹. Conventional anticancer medicines such as surgical resection, chemotherapy, radiation, and molecular targeted therapy are utilised to effectively treat early-stage tumours, but are ineffective for advanced-stage disease. Cancer immunotherapy, which activates the host's immune system, has the potential to prevent cancer recurrence and extend the survival duration of end-stage patients². Cancer immunotherapy was initially studied over a century ago, when Dr. Coley proposed activating the immune system to produce an antitumor response by injecting "Coley's Toxins" into excised sarcoma sites. Since then, many efforts have been made to better understand the immune system's role in cancer progression. Rosenberg demonstrated long-term cancer regression with a high-dose bolus IL-2 injection for metastatic melanoma, earning FDA approval as the first immunotherapy for human cancer³. Cancer immunotherapy is fundamentally changing the discipline of oncology, both theoretically and practically. Clinical studies, particularly those utilising antibodies that induce immune checkpoint blockade (ICB), have definitely demonstrated that cancer can be treated very well without drugging cancer cells. Indeed, immunotherapy can provide better outcomes than earlier standards of care, such as molecular targeted therapy or cytotoxic chemotherapy. As a result, whereas most treatments throughout the last century have concentrated on treating cancer as a cell-autonomous disease, there is a growing interest in understanding the tumour immunological microenvironment (TME) and how it

influences the response to therapy⁴. Nanomedicines have achieved tremendous progress in tumour therapy during the last three decades. Since the first liposomal doxorubicin (Doxil) was licensed by the US FDA for the treatment of ovarian cancer, dozens of nanomedicines have been approved for the clinical treatment of tumours around the world. Nanomedicines preferentially accumulate in solid tumours due to unusually leaky vasculature and defective lymphatic drainage within the TME, which are well-known as the increased permeability and retention (EPR) effect⁵. Nanotechnology-based goods can effectively elicit immune system responses for each characteristic. Vaccines, chimeric antigen receptor T-cell treatment, immune checkpoint blockade, and cytokine injection are among the most recent cancer immunotherapies to be developed. Cancer vaccines, for example, are designed to improve cancer antigen presentation in dendritic cells (DCs), hence increasing the robustness of effector T-cell proliferation. Several immunological approaches have been fully detailed, including immunotherapies and vaccinations, which stimulate immune systems (both adaptive and innate) at the single-cell level. As we all know, one of the most important uses of nanotechnology-based scaffolds and nanoparticles is targeted drug administration, and using this three-dimensional system for cancer therapy could result in a substantial revolution in cancer treatment methods (Fig. 1)⁶.

With the advancement and extension of research, nano-immunotherapy has emerged as a new hotspot and trend in recent years. In this review, we summarise recent achievements in nano immunotherapy and concentrate on the mechanisms by which nanotechnology improves the anticancer effects of traditional immunotherapies¹.



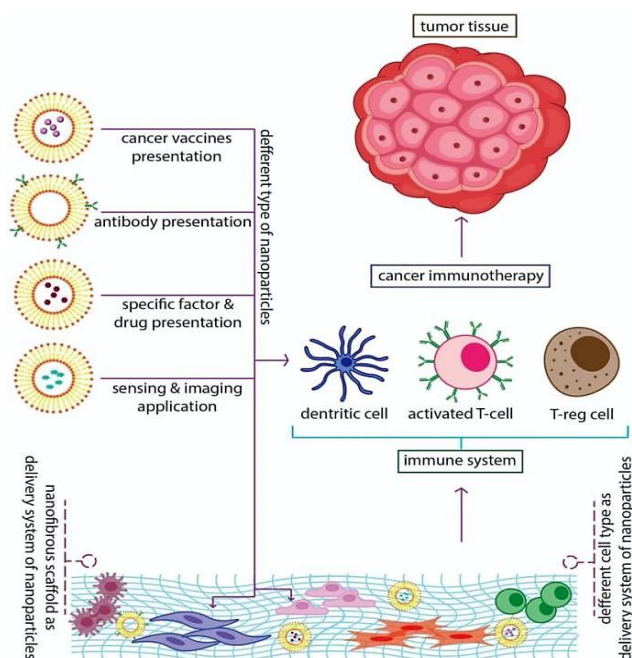


Figure 1: Summary illustration of nanoparticles/nanofibers application for enhancing of the immune system potency against cancer⁶.

TYPES OF CANCER IMMUNOTHERAPY:

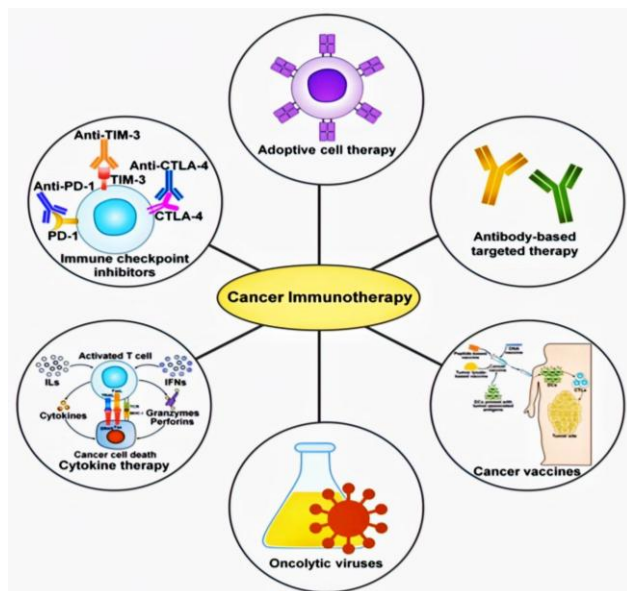


Figure 2: Types of cancer immunotherapy treatments¹.

Current immunotherapeutic strategies used in clinical cancer care mainly include immune checkpoint blockade, adoptive cell therapy, antibody-based targeted therapy, cancer vaccines, oncolytic viruses, and cytokine therapy. PD-1 programmed cell death-1; TIM-3, T cell immunoglobulin and mucin domain-containing protein-3; CTLA-4, cytotoxic T lymphocyte-associated antigen-4. ILs, interleukins; IFNs, interferons; TRAIL, tumour necrosis factor-related apoptosis-inducing ligand; DR4/5, death receptor 4/5; FasL, Fas ligand; TCR, T cell receptor; MHC-I, major histocompatibility complex class I; DC, dendritic cell; CTL, cytotoxic T lymphocyte.¹

1) Adoptive cell therapy:

Adoptive cell therapy (ACT) is a fast-expanding anticancer technique that has showed promise in treating a variety of cancer types. The principle of ACT entails activating patients own immune cells ex vivo and then returning them to the patients to recognise and eradicate cancer cells⁷. Currently, immunotherapy approaches based on immune checkpoint inhibitors, tumour vaccines, and adoptive cell therapy (ACT) have transformed cancer treatment. Immunotherapy has an advantage over the three traditional therapies (surgery, radiotherapy, and chemotherapy) in that it can stimulate the immune system to permanently eliminate residual or disseminated tumour cells and restore immune function that has been compromised by radiotherapy and chemotherapy⁸. Genetic manipulation of blood-derived T cells has been demonstrated to be useful in the treatment of several haematological malignancies, but only modest action has been reported in solid tumours. The use of non-genetically modified, polyclonal TILs that target several epitopes has yielded the most promising clinical results to far⁹.

2) Antibody-based targeted therapy:

Antibody-based targeted therapy has received more attention than other immunotherapeutic techniques for cancer treatment because to antibodies' wide ability to target specific antigens. Antibodies and antibody fragments can target specific antigens produced by cancer cells and induce apoptosis¹⁰. The immunoglobulin (Ig) fold consists of two disulfide-linked antiparallel β -sheets with projecting β -turns. Both the variable and constant domains of the antibody molecule are Ig folds. Three β -turns in variable domains act as complementarity determining regions (CDRs) for hypervariable amino acid sequences. The IgG1 molecule is the most frequent format for natural and synthesised human antibodies¹¹. Antibodies are Y-shaped proteins created by the immune system to protect the host from foreign invasion. When an antigen enters the body, both the innate and adoptive immune systems get activated. In the initial stage, macrophages begin to phagocytose foreign material¹². Antibody-based immunotherapies are a type of therapy that is now being studied in clinical trials to treat acute myeloid leukaemia (AML)¹³.

3) Cancer vaccines:

Cancer vaccines have been under extensive clinical investigation for 40 years, with just a few successes in extremely limited situations. William Coley made the first successful efforts to harness the immune system against cancer in 1891, when he injected live and heat-killed bacteria into bone and soft tissue sarcomas and saw tumour decrease in some patients¹⁴. RNA-based Cancer Vaccines There are numerous methods for administering mRNA vaccines. One of these is the so-called "naked" mRNA vaccination, which involves injecting mRNA directly into a buffer without using a carrier. Nucleic acid can be given via lipoplexes or lipid nanoparticles to improve mRNA stability

and protect it from destruction¹⁵. Cancer vaccination, also known as cancer immunisation or cancer immunotherapy, is a treatment technique that stimulates the immune system to recognise and attack cancer cells¹⁶. DNA-based vaccines contain genetic material aimed against cancer antigens, which, when administered to a patient, cause a specific immune response¹⁵.

4) Oncolytic virus:

Viruses were previously connected with the evil demon. However, oncolytic viruses (OVs) are like noble angels in that they can rescue lives. Oncolytic virotherapy is a new cancer treatment technique that selectively replicates and destroys tumour cells while leaving normal cells unharmed¹⁷. Oncolytic viruses (OVs) are organisms that can recognise, infect, and lyse various cells in the tumour environment, with the goal of stabilising and slowing tumour progression. They can have a natural affinity for cancer cells or be genetically engineered to detect specific targets¹⁸. When it comes to infecting cancer cells, OVs are very selective. Without endangering healthy cells and tissues, they preferentially infect and kill cancer cells. Different OVs can infect cells in a number of different ways. To infiltrate host cells, adenoviruses use proteins such as integrins, DSG2, Cluster of Differentiation (CD) 46, and coxsackievirus-adenovirus receptor (CAR) as receptors¹⁹. Through specific targets, including nuclear transcription factors, including human telomerase reverse transcriptase, prostate specific antigen, cyclooxygenase-2, osteocalcin, and surface markers like prostate-specific membrane antigen, folate receptor, CD20, endothelial growth factor receptor, and Her2/neu, which are compounds produced by the tumour cells, OVs can infect aberrant cells¹⁸.

5) Cytokine therapy:

By inducing tumour antigen expression, antigen presentation, immune cell priming and activation, effector immune cell recruitment and infiltration to cancer, and cancer death in the tumour microenvironment (TME), cytokines impact the entire cancer immune cycle²⁰. There are currently around 130 identified cytokines, which are very tiny signalling proteins with MW<30 kDa that are typically glycosylated and produced by a wide range of cells, including immune system cells, epithelia, endothelia, and stroma. With their autocrine, paracrine, or endocrine functions, cytokines are important regulators of the immunological and inflammatory responses that can either stimulate or inhibit cellular activity in infection, innate and adaptive immunity, autoimmunity, inflammation, and cancer²¹. Through a variety of ways, cytokine treatment can lead to the destruction of tumours. On the one hand, the cytokines directly affect the cancer cells, preventing them from proliferating by triggering apoptosis, inhibiting angiogenesis, and altering their differentiation. On the other hand, the injection or inhibition of particular cytokines that disrupt the corresponding signalling pathways might initiate the anti-cancer immune response²².

6) Immune checkpoint inhibitors:

The purpose of immune checkpoint inhibitors (ICI) is to enhance anti-tumor immune responses by blocking inhibitory signals of T cell activation. ICT has provided long-term clinical advantages in a large group of patients with diverse tumour types since its effective launch as a treatment for unresectable or metastatic melanoma in 2011, including cure in a fraction of patients²³. During the body's immune response, immune checkpoints a set of molecules expressed on immune cells that can adjust the level of immune activation serve as gatekeepers to limit the immune system from becoming overactive²⁴. ICIs are frequently employed in conjunction with other therapeutic approaches, such as radiation therapy, chemotherapy, or surgery, in order to increase the success of cancer treatment by eliciting an effective anti-tumor response. ICIs' ultimate goal is to improve patient outcomes by using the immune system's powerful effector capabilities to fight cancer²⁵. The cancer immunosurveillance concept, which postulated that the immune system might identify and eradicate early cancer cells, served as the foundation for the development of current ICIs. Although the clinical utility of previous cytokine-based immunotherapies, such as interleukin-2 and interferon alpha, was hindered by lack of specificity, resulting in low efficacy and high toxicity, the goal of using the host's own immunity to identify and eradicate cancer cells as "foreign" has been pursued for decades²⁶.

CANCER IMMUNITY CYCLE:

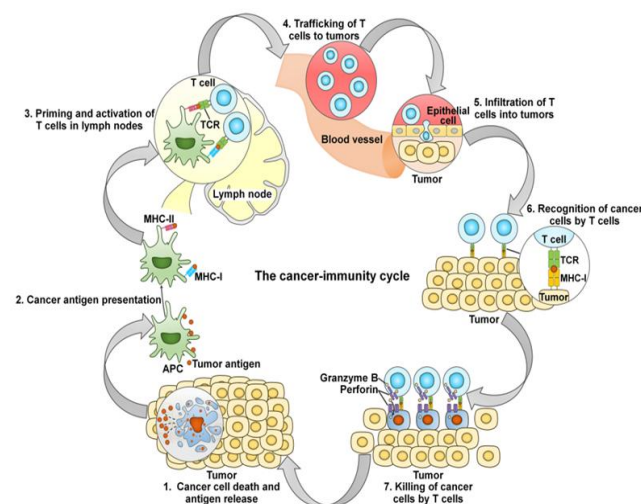


Figure 3: The cancer-immunity cycle¹.

The cancer-immunity cycle comprises a series of functional stepwise events that are required for immune-mediated control of cancer growth. The cancer-immunity cycle is started by the extravasation of antigens from dead cancer cells. Cancer-derived antigens are absorbed by antigen-presenting cells, such as dendritic cells. Antigen-presenting cells then process cancer-associated antigens into smaller peptides. These peptides are displayed on the surface of antigen-presenting cells, along with MHC molecules. Afterwards, these cells move to the draining lymph node, where they present cancer-specific antigens to T cells.

Effector T cells are primed and activated after recognition of peptide/MHC complexes by T cell receptors. Activated T cells migrate to the tumour through the circulation, infiltrate into the tumour and destroy cancer cells via the perforin-granzyme pathway. Once cancer cells die, additional neoantigens are released to initiate a new round of the cancer-immunity cycle. APC, antigen-presenting cell; MHC-I, major histocompatibility complex class I; MHC-II, major histocompatibility complex class II; TCR, T cell receptor ¹.

NANOPARTICLE APPROCHES TO IMPROVE IMMUNOTHERAPY:

1) Antigen and Adjuvant Delivery:

Adjuvants are any of a number of substances that, when combined with vaccine antigens, increase the immunogenicity of vaccinations. Synthetic tiny molecule chemicals, intricate natural extracts, and particle matter are examples of adjuvants ²⁷. Despite the aforementioned developments in adjuvant development, the broad definitions and intricate mechanisms of adjuvants have resulted in a lack of systematic generalisation and summary of their action mechanisms. Furthermore, it is challenging to match and build suitable adjuvants for certain vaccines because of the lack of systematic and thorough knowledge of the mechanisms, traits, immunological efficacy, and application scenarios of the current adjuvant platforms ²⁸. Following the uptake and processing of antigens, the antigen peptides-major histocompatibility complexes (MHC) that are displayed on the surface of APCs are known as antigen presentation signals ²⁹. Co-stimulatory signals include inflammatory cytokines (e.g., IL-6, IL-10, IL-12, and TNF- α) and co-stimulatory molecules (e.g., CD40, CD80, and CD86) expressed on the surface of APCs. An enhanced adaptive immune response can result from the high activation of naive T cells caused by the generation of these two signals ³⁰.

a. Vaccines without adjuvants induce modest production of T helper-polarizing cytokines, antibodies, and activated T cells.

b. In contrast, vaccines with adjuvants promote the maturation of more APCs, increase the interaction between APCs and T cells, promote the production of greater numbers and more types of T helper-polarizing cytokines, multifunctional T cells, and antibodies, leading to broad and durable immunity, as well as dose and antigen savings ²⁷.

2) Targeting Antigen-Presenting Cells Delivery:

The immunological effects of antigens against different illnesses or malignancies can be enhanced by a suitable delivery mechanism. The innate and adaptive immune responses are connected by antigen-presenting cells (APCs), which are specialised to capture and process antigens in vivo. One promising tactic for eliciting strong immune responses is the functionalisation of vaccine delivery systems with targeting moieties to APCs ³¹. A vital component of the innate immune system, antigen-presenting cells (APCs) include B lymphocytes, dendritic cells (DCs), and macrophages. They are crucial for both starting and controlling the adaptive response ³². Nanoparticles (NPs) are frequently employed as vaccine delivery vectors, and the advancement of delivery systems has expedited the deployment of vaccines ³³. By combining NPs with targeting molecules, their capacity to target particular tissues or cells can be modified, enhancing the delivery of particular immune cells and lowering the buildup of cargo in organs that are not the intended targets. NPs that are engineered to react to certain stimuli can control antigen trafficking and release to improve immunological safety and effectiveness ³⁴.

3) Targeted Antibody Delivery:

Antibody-based cancer therapy, including chimeric antigen receptor T (CAR-T) cells, antibody-drug conjugates (ADCs), immune checkpoint inhibitors (ICIs), angiogenesis inhibitors, and multi-specific antibodies, has become one of the most effective therapeutic approaches. Numerous drug delivery systems have been created to enhance the bioavailability, simplicity of distribution, and decreased toxicity of antibodies in order to boost their therapeutic efficacy ³⁵. A monoclonal antibody (mAb) is usually covalently bonded to a cytotoxic medication by a chemical linker to form an antibody–drug conjugate (ADC). In order to accurately and efficiently eradicate cancer cells, it combines the benefits of highly specific targeting with a highly effective killing impact. This has made it a hotspot for anticancer drug research and development ³⁶. Chemotherapy with cytotoxic medicines has proven to be quite effective in treating cancer. However, off-target cytotoxicity frequently results in severe side effects that impair patients' quality of life and even endanger their lives. Therefore, mAb-conjugated highly cytotoxic drugs have adequate affinity and specificity for tumour antigens ³⁷.

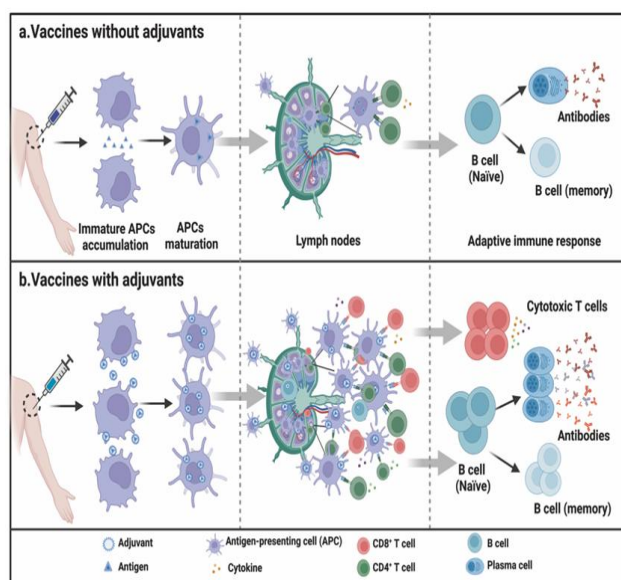


Figure 4: Adjuvants enhance the immunogenicity of vaccines.

4) Gene Delivery:

One of the most innovative medical innovations created using DNA recombination and gene cloning technology is gene therapy³⁸. The novel method of using genes to prevent or treat any illness is called gene therapy. By introducing a gene into a patient's cell, gene therapy may enable medical professionals to treat a condition without the need for medication or surgery³⁹. A number of gene therapy strategies are being investigated by several scientists and medical professionals, such as:

Substituting a healthy gene for a disease-causing mutation.

"Knocking out," or deactivating, a gene mutation that isn't working correctly.

Adding new genes to the cells to guard against illnesses⁴⁰.

Nanoparticles allow for controlled encapsulation and delivery of genetic material, offering enhanced therapeutic efficacy while minimizing off-target effects⁴¹.

NANOPARTICLE'S STRUCTURES FOR CANCER IMMUNOTHERAPY:

1) PLGA:

In drug delivery systems, poly (lactic-co-glycolic acid) (PLGA) is a commonly used biodegradable and biocompatible polymer, especially for encapsulating therapeutic compounds with low permeability and solubility⁴². The release behaviour of sustained-release preparations is directly impacted by the degradation behaviour of PLGA polymer, which serves as a nano drug laden nanoparticle carrier. Thus, the release degree of sustained-release medications can be modified by modifying the pertinent parameters influencing the PLGA breakdown behaviour⁴³. The ratio of PLA (polylactic acid) monomer to PGA (polyglycolic acid), relative molecular weight (Mw), crystallinity, degradation environment (temperature, pH, etc.), drug type, and preparation type following matrix encapsulation of the drug are the factors influencing the degradation behaviour of PLGA⁴⁴. Because they are biocompatible and biodegradable, PLGA-based nanoparticles (NPs) can effectively treat tumours by sustaining the release of different antigens and anticancer drugs⁴⁵.

2) Liposomes:

Phospholipids, specifically glycerophospholipids and sphingomyelins, make up the majority of liposomes. Vesicles with a size range of 20 nm to 2.5 μ m are called liposomes. One or more concentric or non-concentric membranes may make up these structures. Acute parameters influencing drug encapsulation efficiency, clearance, and half-life in circulation include vesicle size and number of bilayers⁴⁶. To precisely associate a certain nanoparticle feature with biological processes, liposomes and nanoparticles (NPs) must be physically characterised⁴⁷. Numerous challenges affect the viability of liposome drug delivery systems for the treatment of cancer in clinical settings. The efficacy and lifespan of liposomes are

diminished by instability issues, such as drug leakage during storage⁴⁸. Being nanomaterials, liposomal DDS have several fundamental properties, including a high surface-to-volume ratio, improved biocompatibility, and heightened permeability through biological barriers⁴⁹.

3) Dendrimers:

Targeting tumour cells, regulating the release of anticancer drugs, and integrating anticancer techniques are some of the special uses for dendrimers, which are nanomaterials with special features. The unchecked growth of abnormal cells is the hallmark of cancer, a malignant disease. Proto-oncogenes, which encode proteins associated in the growth and differentiation of tumour cells, as well as tumour suppressor genes, which encode proteins that produce apoptosis and inhibitory signals for cells in need, are imbalanced or damaged, which promotes the advancement of cancer⁵⁰. Complete dendrimers are those that finish in amine groups (-NH₂) or hydroxyl groups (-OH), whereas half-dendrimers are those that end in carboxyl groups⁵¹. By enhancing the stability, solubility, and bioavailability of medications, dendrimers are among the most widely used nanomaterials that are readily available and provide a number of advantages in the chemotherapy-based treatment of cancer. Because they are highly branched polymers, conjugating and encapsulating medications is easy⁵². The passive targeting of cancer is made possible by the nanoscale characteristics of dendrimers, which allow the differentiation of intrinsic metabolic differences between cancer cells and healthy cells. Furthermore, dendrimer surfaces are readily functionalised to enhance selectivity and facilitate active cancer targeting. Dendrimers may therefore be investigated as intelligent nanocarriers for chemotherapy⁵³.

4) Virus-Like Particles:

Particles that self-assemble due to the expression of proteins encoding capsids, cores, or envelopes of viruses, or even preparations of monolayered particles formed from a multilayered virus, are referred to as viral-like particles (VLPs)⁵⁴. Choosing the right antigen for effective in vivo delivery is one of the most important problems that must be solved for a VLPs-based cancer antigen delivery platform. Tumour antigen loading gives VLPs the ability to trigger immune responses specific to tumour antigens, giving the immune system the "aiming ability" to target cancer cells that have tumour antigens with precision⁵⁵. VLPs: Chemical and Biological Features It is possible for various cells (such as bacteria or yeast) as well as plants, insect or mammalian cell lines, and other organisms to produce virus-like particles. The primary distinctions between VLPs made in bacteria and human cells are related to post-translational changes that are crucial for the immunological response, such as glycosylation and phosphorylation. Compared to mammalian cells, bacteria and insect cells produce more VLPs⁵⁶. Viral antigen presentation on MHC class I and MHC class II molecules results from VLPs' effective interaction with dendritic cells (DCs), the most powerful antigen-presenting cells. To



activate the antigen-specific CD4+ T helper and CD8+ cytotoxic T cells, activated DCs go to lymph nodes. Specifically, when persistently infected cells present the peptide MHC-I complex, CD8+ T lymphocytes are activated and begin to exhibit their cytotoxic activity⁵⁷.

5) Hybrid Particles:

There are several methods for creating hybrid nanoparticles that contain both organic and inorganic materials. Systematic tweaking of hybrid nanoparticles' features for combination cancer therapy should be possible due to their modular design, which enables the integration of numerous organic and inorganic components⁵⁸. Liposomes and polymeric nanoparticles are examples of drug nanocarriers. The phrase "lipid polymer hybrid nanoparticle" refers to the fact that they can be combined to create a potent hybrid nanoparticle that can be employed for a variety of therapeutic and diagnostic applications. These hybrid nanoparticles outperform conventional nanocarriers by combining biocompatibility, a high drug-loading capacity, and controlled release characteristics⁵⁹. Gel's interactions with cancer tissue give it strong adhesive qualities, and its hybridisation with nanoparticles lengthens the retention period in the tumour. Targeted medication administration can be facilitated by extending the retention duration of the nanoparticles by injecting the gel–nanoparticle hybrid directly into the cancer site or by causing it to form a hydrogel within the tumour⁶⁰.

CONCLUSION

Cancer immunotherapy has revolutionized the field of oncology, and nanoparticle-based approaches have shown great promise in enhancing the efficacy of these therapies. By targeting specific cells and delivering therapeutic agents, nanoparticles can improve the outcomes of cancer treatment. The various types of nanoparticles, including PLGA, liposomes, dendrimers, virus-like particles, and hybrid particles, offer a range of opportunities for cancer immunotherapy. Further Study is needed to fully realize the potential of nanoparticle-based approaches in cancer treatment. However, the progress made so far is encouraging, and it is likely that nanoparticles will play a significant role in the future of cancer therapy. With continued advancements and innovations, nanoparticle-based cancer immunotherapy may become a powerful tool in the fight against cancer.

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